

FURTHER STUDIES ON THE INFLUENCE OF SUCKLING

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SEVEN FIGURES

It has recently been shown, in experiments on rats and mice (Selye, '34), that cessation of secretion and involution of the mammary glands which follow removal of the litter are due, not to the accumulation of milk within the gland, but rather to the withdrawal of the stimulus of suckling; and it has also been shown that the application of this stimulus to normal adult females leads to development of the mammary glands and eventually to milk secretion (Selye and McKeown, '34 a), and to prolonged, almost continuous dioestrus, with changes in the uterus, ovaries, and hypophysis (Selye and McKeown, '34 b) which led us to describe the condition as 'suckling pseudopregnancy.' It seemed desirable to extend these studies, and to reconsider certain current ideas of the physiology of lactation, in the light of the emphasis now placed on the influence of the act of suckling. It is well known, for instance, that lactation cannot be maintained indefinitely, even though the milk is continually removed from the mammary gland. Thus in dairy cattle, even if the animals are regularly milked, the milk production eventually decreases, and involutionary changes in the gland have been made in other species.

In a group of eight lactating mice, we maintained the stimulus of suckling for a considerable period beyond the usual span by replacing the growing litters from time to time by very young ones; in this way it was possible to be

sure that a strong nursing stimulus was continually present. It was observed that, after about 8 weeks, such animals were unable to nourish their young, and from this time onward the litters were exchanged daily with the actively nursing litters of normally lactating mothers, so that death from inanition was averted. Even after a very long period (in two cases as long as 68 days) normal oestrous cycles had not reap-

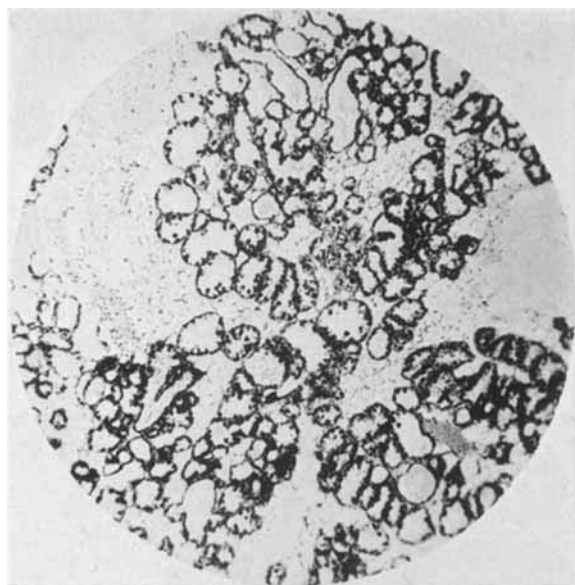


Fig. 1 Low magnification of the mammary gland of a mouse taken during the fourth week of lactation. Excrecences of epithelial cells invade the lumen of the acini.

peared. The vaginal smears indicated that the first recurrence of oestrus occurred in the third week after parturition, that is at the time at which it has usually been reported in the normal course of lactation. This oestrous period was followed, however, not by normal cycles, but by successive periods of dioestrus each of from 12 to 17 days' duration, separated the one from the other by a single appearance of oestrus. In a similar experiment, Crew and Mirskaia

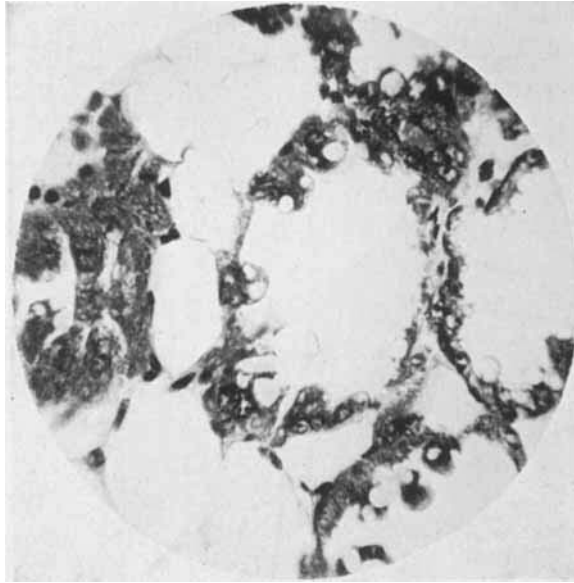


Fig. 2 High magnification of an acinus in the mammary gland shown in figure 1. Several cells are missing from the epithelial lining.

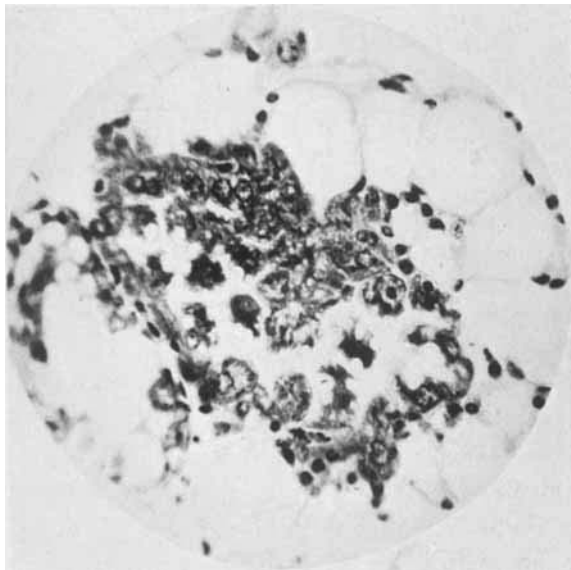


Fig. 3 High magnification of another acinus in the mammary gland shown in figure 1. Numerous cells lie free in the lumen.

('30) failed to observe this phenomenon, since they abandoned their studies at the first reappearance of the cornified vaginal smear; and Parkes ('26) allowed his animals to copulate and become pseudo-pregnant at each recurrence of oestrus.

In these same animals, we followed the changes in the mammary gland by taking biopsy specimens for histological

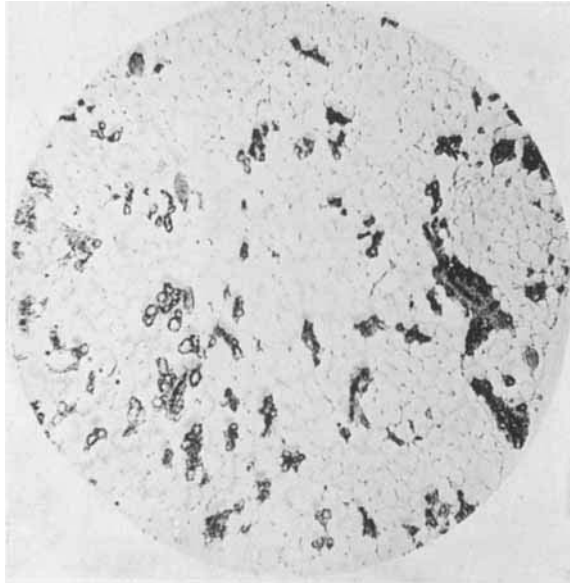


Fig. 4 Low magnification of the mammary gland of a mouse on the forty-second day of lactation. The parenchyma has undergone complete involution and only the duct system is left.

study. Degeneration was quite marked in some cases by the end of the fourth week, and in all at least the beginning of retrogression could be seen at this time. Figure 1 illustrates the histological picture of this type of involution, which is quite characteristic and distinct from the picture of physiological involution after weaning. The first sign of degeneration is the budding-out of cells or cell-complexes into the lumina of the acini; this is often so marked that the gland assumes a papillomatous appearance. At this stage all

the epithelial cells of the acini become very markedly basophilic, so that the nucleus is hardly distinguishable from the cytoplasm. At a later stage, the epithelial cell buds are shed and lie free in the lumen (figs. 2 and 3) some weeks later still, the whole parenchyma of the gland disappears, and only the duct system remains (fig. 4). We are under the impression that the budding of cells into the lumen is

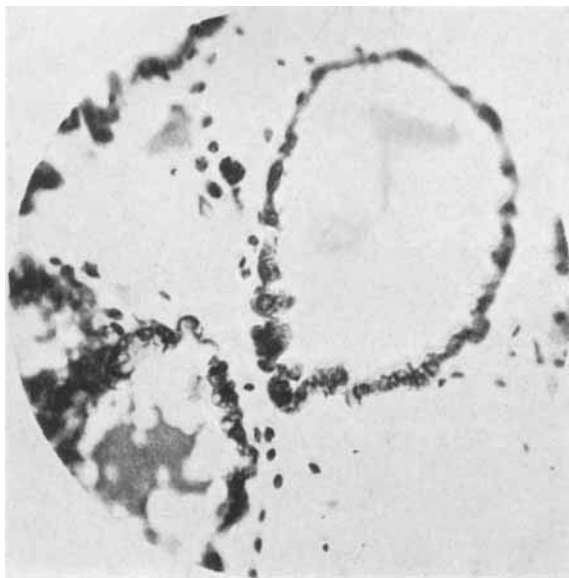


Fig. 5 High magnification of an acinus during the fourth week of lactation. Some of the epithelial cells extrude toward the stroma.

sometimes accompanied by a budding toward the stroma, that is in the opposite direction; for in degenerating glands we have frequently observed cells extruding from the epithelial lining into the interacinar spaces (fig. 5), and cells and cell-complexes of epithelial type and possessing basophilic cytoplasm may often be found in the stroma (fig. 6). Vacuoles eventually appear in these free epithelial cells, which come to assume the appearance of fat cells (fig. 7).

It is evident from this experiment that although the stimulus of suckling, if vigorously applied, can maintain a condition of almost continuous dioestrus for long periods in normally lactating mice, it cannot keep the mammary gland in a state of full development and function indefinitely.

We have described elsewhere (Selye and McKeown, '34 a and b) 'suckling pseudo-pregnancy,' induced by applying a vigorous nursing stimulus to normally cyclic adult female

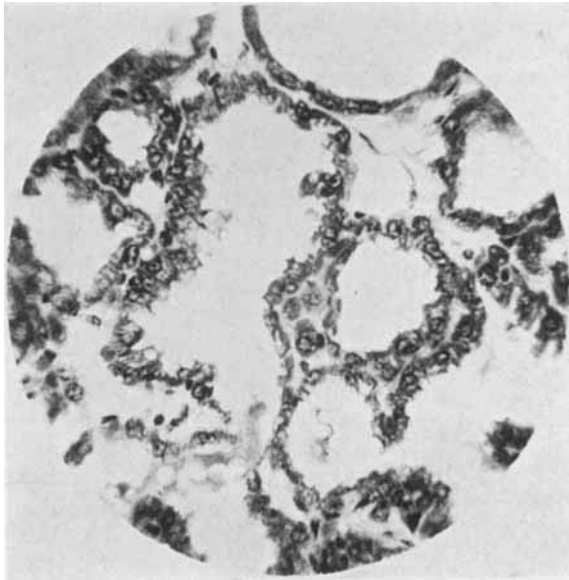


Fig. 6 Another section of the mammary gland shown in figure 5. In the center of the field several cells of epithelial type similar to the cells lining the wall of the acini are seen in the stroma.

rats and mice. In a recent repetition of these studies, on a group of six normally cyclic mice, we noted carefully when the act of suckling actually began. Biopsy specimens of the mammary glands taken at intervals thereafter showed that duct development had just begun on the third day; the gland was quite well developed on the fourth day in half the animals, and on the eighth day in all. The mice were sacrificed on the twelfth day, by which time one out of the six had actually commenced milk secretion.

We have pointed out (Selye and McKeown, '34 b) that 'suckling pseudo-pregnancy' differs from the 'copulation pseudo-pregnancy' produced by sterile coitus in that the former does not follow inevitably from one single stimulus, but requires that the nursing stimulus be constantly maintained. A further point of distinction deserves mention at this time: we agree with previous workers that sterile copulation is usually followed by proliferation of the mammary

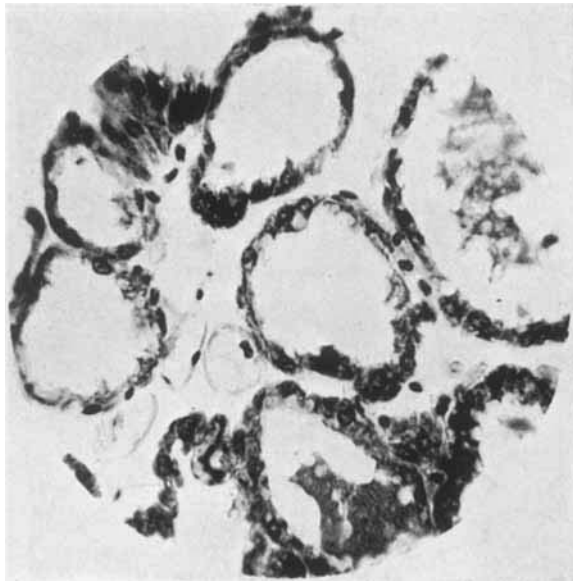


Fig. 7 Vacuolized cells in the stroma of the mammary gland shown in figures 5 and 6. Numerous intermediate stages between the typical epithelial cell and this vacuolized cell may be found in the stroma at this stage.

gland, in the rat; but while those who have studied this phenomenon in the mouse (Turner and Gomez, '33; Cole, '33) apparently regard it as regularly present, we have been unable to convince ourselves that this is the case. In a series of ten cyclic female mice which were mated with vasectomized males, and which all showed the usual prolongation of dioestrus, biopsy specimens of the mammary glands showed evidence of proliferation in only two instances. It

was found, however, that if the females were left with the sterile males for several weeks, mammary development did occur more frequently. We have never failed to observe mammary development in suckling pseudo-pregnancy in rats or mice.

Hypophysectomy performed during normal lactation leads promptly to cessation of secretion and involution of the glands both in rats (Collip, Selye and Thomson, '33) and in mice (Selye, Collip and Thomson, '33). We therefore hypophysectomized a rat and a mouse on the sixth day of suckling pseudopregnancy, when histological examination of samples from the mammary glands showed that considerable development had taken place; further samples, taken 5 days later, showed complete involution, although the suckling stimulus had been actively maintained in both animals; the corpora lutea also showed retrogressive change.

It is well known that ovariectomy, on the other hand, has no influence upon normal established lactation. We were therefore interested to know whether the ovary was necessary for the changes which we had observed to be brought about under the influence of the suckling stimulus in the normal animal. Four adult, normally cyclic mice were ovariectomized and supplied with actively nursing litters, which we maintained by exchanging them daily for the young of a normally lactating mother. The animals were sacrificed after 13 days, and the sections showed that the mammary glands and uterus were not affected by the suckling stimulus, but were identical with the organs of three castrate controls which had not been given litters.

It has previously been shown (Selye, '34) that if, in lactating animals, the escape of milk is prevented by transcision of all galactophores, the continued stimulus of suckling prevents involution of the mammary glands from occurring as rapidly as it does when the nipples are destroyed or the young removed. This delay in involution does not depend upon the ovaries; for in five normally lactating mice in which we performed bilateral ovariectomy as well as transection of

all milk ducts, we found by examination of mammary gland specimens from time to time that involution occurred more slowly than in four controls which were weaned and ovariectomized on the same day of lactation. From this and the preceding experiment, we conclude that the nervous stimulus of nursing probably exerts its trophic effect on the lactating mammary gland by way of the pituitary. Additional evidence for this theory has been discussed elsewhere (Selye, Collip and Thomson, '34).

Although transicion of the galactophores does not lead to rapid involution if the suckling stimulus is maintained, nevertheless, the pressure of the accumulating milk eventually leads to degenerative changes within the mammary gland (Selye, '34). This observation was confirmed on a fresh series of six lactating mice, in which the operation was performed the day after parturition. When the pressure of milk had at least produced atrophy (much later than in the weaned controls), the glands were unresponsive to the stimulus of suckling. At a still later date, however, it was observed that the responsiveness of the glands to the stimulus of suckling was to some extent restored, although the degree of development attained was markedly inferior to that seen in normal mice when the stimulus of suckling is first applied.

SUMMARY

1. If the stimulus of suckling is maintained by frequently supplying fresh litters to normal lactating mice, active lactation may continue for about 2 months, after which an unusual type of involution of the mammary gland occurs. In spite of this involution, normal oestrous cycles do not reappear, the animals showing successive long periods of dioestrus, each lasting from 12 to 17 days.

2. The application of the suckling stimulus to normally cyclic mice leads to inhibition of oestrus to development of the mammary glands. This response is unobtainable in mice previously ovariectomized, and cannot be maintained after hypophysectomy.

3. Copulation pseudopregnancy, unlike suckling pseudopregnancy, is not always attended by mammary development in the mouse.

4. The stimulus of suckling maintains secretory activity in the mammary gland for some time after escape of milk is prevented by transcision of all galactophores; this maintained activity may be seen even in the absence of the ovaries.

5. The pressure of the accumulating milk after transcision of galactophores eventually causes atrophy of the glands, which then become unresponsive to the stimulus of suckling; later, however, they regain in part the capacity to respond to this stimulus.

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